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The
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AND A
Related Saprophyte

BY

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THE CHESTNUT BLIGHT FUNGUS AND A RELATED SAPROPHYTE

By P. J. and H. W. ANDERSON

INTRODUCTION.

When the Pennsylvania Chestnut Tree Blight Commission undertook to determine the extent of the blight disease in Western Pennsylvania, they were confronted with a puzzling condition of what was apparently the blight in a few of the extreme south western counties. In these localities a fungus was found quite commonly on the chestnut trees, which superficially, could not be distinguished from the true blight fungus, but it was apparently causing no serious injury to the trees. Aside from the fact that this fungus was usually found only on stumps and dead parts of the trees, one other peculiarity was noticed. One of the most characteristic features of the true blight is the presence of fan-shaped areas of fungous mycelium in the bark on the scalloped advancing edge of the canker. These areas are entirely absent in the bark of the trees infested by the "Western or Connellsville Fungus"—by which name we shall designate the fungus occurring in these southwestern counties. Mr. J. K. Hibbs, supervisor of this southwestern district, being in doubt as to the identity of the fungus, submitted specimens to all the leading pathologists who have concerned themselves with this disease. They uniformly agreed that this fungus was the true blight organism, *Diaporthe parasitica*, as we shall call it in this paper. Microscopic examinations were made, but if any differences were noticed, they were ascribed to local conditions, immaturity of the specimens or various other causes. Many theories were advanced to explain its peculiar behavior in this district. Some believed that it was due to the large amount of coal smoke in the atmosphere of that region. Others thought the trees there were more healthy and therefore more resistant. Still others considered it a saprophytic strain of *Diaporthe parasitica*, while some advanced the theory that this was the saprophytic progenitor of the deadly eastern parasite. No light

was thrown on the relation of these fungi at the Harrisburg conference in February of last year, although the existence of a so-called saphrophytic strain was mentioned by several speakers.* The uncertainty about the relation of these forms has given rise to much confusion as to the extent of the blight.

With this puzzling condition of affairs confronting the Commission, it was thought best to make a careful study of the western problem and for this purpose a field laboratory was located at Connelville, where the so-called western fungus was quite common. The results of the investigation carried out at this laboratory are set forth in the following pages.

OBSERVATIONS ON THE NATURAL HABIT AND HABITAT OF THE FUNGUS.

In external macroscopic appearance this fungus resembles *Diaporthe parasitica* in all its stages and there seems to be no way in which it can be distinguished from this fungus in the field, except by the absence of the areas of fan-shaped mycelium. On young bark the western fungus develops small, scattered orange pustules under the epidermis. Areas of orange colored mycelium are often found throughout the thin bark. The pycnidia are formed in the pustules beneath the epidermis and the spore horns develop singly, pushing out from the top of the conelike pustules. The pustules on older bark are much larger, often reaching three or four millimeters in diameter. These occur as a rule in the crevices of the bark but are not confined to this region, being especially well developed on the bark of the callus at the edge of a rotted area or at the base of the stump on the exposed roots. The pustules vary greatly in color from a light yellow to almost black, a deep orange being the most common color observed. In the coke-oven region, old pustules are usually black externally on account of the smoke. The stroma is a light yellow color and pulverulent in the young condition, darkening and hardening with age. A number of pycnidia may be formed in each of these stromata. Perithecia may be found at any season of the year and are developed in the same manner as in *Diaporthe parasitica*. On the inner surface of the bark which has separated from the wood and on the wood protected by the bark, single pear-shaped pycnidia are found, in general appearance similar to the eastern fungus. Small reddish, flattened, single pycnidia are also developed on the top of stumps or on the end of logs several inches in from the edge of the bark. They are also formed on the cut edge of thick bark, especially when this is somewhat shaded.

*Report of the Chestnut Tree Blight Conference, Feb. 1912, pages 47 and 80.

Special attention was given to the habitat of this fungus since the question of its parasitism would be largely based upon these observations. It was usually found growing on stumps from which the trees had been removed one or two years, or upon fallen logs of about the same age. It was seldom found on trees or stumps which had been dead for a longer period. Many careful examinations of the coppice at the base of an infected stump were made since this was a common point of infection by *Diaporthe parasitica*, but always with negative results. Die-back conditions and cankers produced by other fungi and insects are common on the coppice in this section of the State. The western fungus was often isolated from such areas, but was never found to be the primary cause of the diseased conditions. It was also found on thick bark of old trees where no injury could be found, but in no instance had it penetrated into the cambium. Very often when it was found on the apparently healthy trees, cutting into the bark would show that the borers had been at work and had killed the tissue below the area infected with the fungus. There has been no case during all the investigations where the western fungus was found causing the death of a tree. Aside from the chestnut it has been found on several species of oaks, among these the chestnut oak being its most common host.

MICROSCOPIC EXAMINATIONS AND COMPARISON WITH THE EASTERN FUNGUS.

A microscopic examination of the western fungus revealed a number of striking characters by which it could readily be distinguished from *Diaporthe parasitica*. On account of the small size of the conidia, no effort was made to find in these a basis for differentiating the two species. A large number of conidial measurements indicated that the difference between the two species is very slight in this respect.

The examination of the perithecia, asci and ascospores revealed a number of differences, the most marked of which were the size and shape of the ascospores. There was also a very pronounced difference in the length of the asci. In the following table is given the measurements of the asci and ascospores of *Diaporthe parasitica* and the western fungus taken from a number of sources. The measurements here recorded are only a portion of those actually made but indicate the range of the material used.

The Connellsville specimens were collected from various localities about this city and no two of them were obtained from the same tract. The measurements of the Virginia and Tennessee specimens were included under the Connellsville fungus since it has been shown from our cultural and microscopic examinations that there is no difference between the fungi collected from these various localities. All measurements were made with a 1-12 oil immersion objective.

TABLE I.

Showing the relative size of the ascospores of the Connellsville fungus and *Diaporthe parasitica*.

Connellsville Fungus.

Locality.	Diameter.		Length.	
	Number of measurements.	Average diameter.	Number of measurements.	Average length.
Connellsville No 50, -----	55	2.93	55	6.78
Connellsville No. 51, on oak, -----	61	3.2	61	6.9
Connellsville No. 52, -----	57	2.91	57	6.0
Connellsville No. 55, -----	63	2.93	63	6.5
Connellsville No. 56, -----	30	3.1	30	7.22
Greene Co., Pa., -----	39	3.0	39	6.8
Lynchburg, Va., -----	50	3.1	90	6.9
Erwin, Tenn., -----	40	3.1	40	6.9
Fauquier Co., Va., -----	81	3.1	81	6.86
Albermarle Co., Va., -----	50	3.0	50	6.88
Morgantown, W. Va., -----	50	2.4	50	6.98

Diaporthe parasitica.

Mt. Gretna, Pa., -----	62	4.54	50	8.52
Highland, N.Y.,* -----	75	4.40	75	8.8
Somerset, Pa., -----	60	4.4	60	8.2
Connellsville, Pa. M. Gray orchard, imported, -----	25	4.6	25	8.4

*Measured by W. H. Rankin.

TABLE II.

Showing the relative length of the asci of the Connellsville Fungus and *Diaporthe parasitica*.

Connellsville Fungus.

Locality.	Number of measurements.	Average length.
Connellsville No. 50,	50	33.21
Connellsville No. 51, on Oak,	30	35.73
Connellsville No. 52,	51	32.1
Connellsville No. 55,	43	32.0
Connellsville, No. 56,	56	38.05
Greene Co., Pa.,	30	34.7
Lynchburg, Va.,	40	36.7
Erwin, Tenn.,	40	32.4
Fauquier Co., Va.,	60	34.29
Albermarle Co., Va.,	51	32.52
Morgantown, W. Va.,	50	34.72

Diaporthe Parasitica.

Mt. Gretna, Pa.,	35	51.7
Somerset, Pa. No. 77,	60	48.6
Highland, N. Y.,*	75	52.0
Connellsville, Pa. M. Gray orchard, imported,	18	53.6

*Measured by W. H. Rankin.

In general, it will be seen that the ascospores of the Connellsville fungus average 7x3 mikrons, while those of *Diaporthe parasitica* average 8.5x4.5 mikrons. The maximum and minimum sizes are as follows: Connellsville fungus, 8.8-5.7 mikrons; *Diaporthe parasitica*, 9.94-7.1 mikrons. The average length of all asci measured gave 34 mikrons for the western fungus and 51.3 mikrons; for *Diaporthe parasitica*. The maximum and minimum sizes are as follows: Connellsville fungus, 45.5-28.4 mikrons; *Diaporthe parasitica* 58.2-42 mikrons. The contrast in the size here given is striking but even more striking is the difference in the shape of the ascospores.

As shown by the measurements the *Diaporthe* ascospores are much wider in proportion to their length than those of the Connellsville fungus. The relation is about 1:1.9 in the former, and 1:2.7 in the latter. Furthermore, the septa in *Diaporthe* are very evident and a distinct sinus may be seen on the mature spores, while an indistinct septum and a very slight, if any, sinus is the rule in the Connellsville fungus. These characteristics are so evident that a glance at the spores under the microscope by one familiar with the two fungi, is sufficient to distinguish them, provided the ascospores are mature.

Aside from the difference in length, the asci of the two fungi are similar, except that the wall is usually more evident in the western fungus. The perithecia of the western fungus are much smaller than those of *Diaporthe parasitica*. A number of measurements made from specimens collected at Connellsville and from various points in Virginia, gave an average measurement of 346 mikrons in contrast to 490 mikrons, obtained from perithecia of *Diaporthe parasitica* from Mt. Gretna. It was also noticed that the walls of the perithecia of the western fungus were much darker in color than those of the blight fungus.

Since the blight is not found in the southwestern portion of the State where the western fungus is found, there might arise the objection that the measurements obtained above are not comparable, in that local conditions might influence the size of the spores. On a farm a few miles northeast of Connellsville were found some chestnut trees badly infected by the real blight. These were nursery trees which had been planted two years previous, and had not shown signs of the disease until last summer when the winter stage was found. Asci and ascospore measurements from these are given in the tables and show no variation from *Diaporthe parasitica* measurements although taken from the center of the locality where the western fungus flourishes.

ISOLATIONS.

The most successful method of isolating *Diaporthe parasitica* which has been used in the field laboratories of the Pennsylvania Chestnut Tree Blight Commission is this: The outer bark is peeled with a sterile scalpel from over the advancing edge of a young canker and a small piece of tissue just on the line between the healthy and diseased inner bark is transferred to a potato agar slant. One hundred per cent. of pure cultures by this method is the rule in these laboratories. Such a method, however, could not be used to

isolate the western fungus since it was rarely found advancing on the healthy tissue and even where it was found in close proximity, a transfer usually gave several bacterial and fungal contaminations besides the desired organism. In many instances, however, successful isolations were made in this way, since the fungus is a rank grower and the edge of the colony is apt to be pure. Usually, however, other methods had to be used. The most successful of these was the conidial streak. If the specimen showed the fungus to be in the pycnidial stage—summer stage—it was placed in a moist chamber for a few days, and invariably spore horns were pushed out from the stromata. These horns were detached with a wet sterile needle and the free end of the horns streaked on agar slants. Where only the perithecial stage was present, however, the culture had to be made from the ascospores. Two methods of making isolations from the ascospores were used. In the first, the stroma with the enclosed perithecia was removed and the bottom cut off with a sharp sterile scalpel, thus exposing the contents of the perithecia. Then a very minute drop of water was touched to the gelatinous mass of spores and the water containing the spores drawn into a fine capillary tube from which it was blown into a sterile petri dish. Agar was added and the developing colonies isolated. A more successful method, however, was by inducing the perithecia to shoot the ascospores upward on to a sterile agar plate inverted a few millimeters above the ostioles. This method was found to be the most convenient of all. With these four methods it was found possible to isolate the fungus from any kind of a specimen sent in, provided the spores and mycelium were not entirely dead. Isolations were made from specimens collected in over fifty different localities. Most of these were in southwestern Pennsylvania, but a few of them—as elsewhere mentioned—were from Virginia, West Virginia and Tennessee. All of them were identical, however, and need not be discussed separately, i. e., they all showed the same cultural characters. Nor did it seem to make any difference whether the isolation was made from mycelium, ascospores or conidia; they all grew alike. Isolations were made from dead stumps, logs, die-backs, on coppice and apparent cankers on living trees, but all proved to be the same. Neither did the isolations from the oak stumps and logs give different results.

INOCULATIONS.

The final test of the pathogenicity of a fungus is its ability to produce the disease in its typical form when introduced into the host under normal conditions. The importance of making a large

number of inoculations controlled by proper checks was realized early in the work. The methods of making these inoculations were those which had proved most successful in the inoculations with *Diaporthe parasitica* at other laboratories.

When mycelium, either from the tissue or from culture was used, a slit was made under the bark and a piece of the tissue or a portion of the agar with the mycelium growing on it, was introduced into this slit. These are called slit inoculations. When conidia or ascospores are used, these are shaken up in a quantity of water and introduced thus, or the dry spore horns may be used. The point of a heavy knife is thrust obliquely into the bark with the broad side of the blade facing the tree, without removing the point, the knife is pulled downward and away from the tree. Several drops of the spore-containing liquid are then dropped from a pipette into the exposed wound back of the knife blade. The tree quickly sucks up these drops, so that this has proved a very effective method of introducing spores into the living tissue. Between 80 and 100 per cent. successful inoculations have been secured with the true blight fungus by these methods. The following series of inoculations were made at Connellsville.

1. Mycelium of western fungus from tissue.
2. Mycelium of *D. parasitica* from tissue.
3. Mycelium of *D. parasitica* from culture.
4. Mycelium of western fungus from culture.
4. Conidia of western fungus.
6. Conidia of *D. parasitica*.
7. Ascospores of western fungus.
8. Ascospores of *D. parasitica*.

Nearly a thousand inoculations with the western fungus were made at Connellsville together with a few with *Diaporthe parasitica* as checks. On account of the danger of introducing the disease a very limited number of inoculations were made with the eastern fungus, and these were carefully guarded and cut out after they had advanced to the stage where there was no longer any doubt of their power to produce the typical disease. Our inoculations with *Diaporthe parasitica* gave 100 per cent. infection with mycelium from culture and from the tissue and also from ascospore inoculations. The conidial inoculations had given over 80 per cent. infection when they were cut out. In all cases a definite canker with the typical

scalloped edge and the invading fan-shaped mycelial areas developed within a month after the inoculations were made. In a few older cankers left for two months, the infected area extended nearly an inch beyond the edge of the inoculation wound.

The western fungus developed in the dead tissue above the inoculation wound, and within a month had developed pustules upon which spore horns were frequently found, but the growth of the fungus was limited to the area in which the tissue was killed by the inoculation. If it spread beyond this area, it was in the dead bark above the living cambium. There was always a definite even line between the dead and living tissue and no fan-shaped areas of mycelium were present. In all inoculation wounds a healthy callus had formed and in those made three months previous, this growth had almost cloved over the wounds. In some cases bacteria and insects delayed the formation of a callus in some parts of the inoculation, but when these were removed the callus quickly developed.

Inoculations made with ascospores of the western fungus on chestnut oak developed as on the chestnut, forming spore horns on the dead area above the wound. Reisolations were made from these spore horns, proving the fungus to be the same as that used in the inoculations.

These inoculation tests were confirmed by the results obtained at the Charter Oak laboratory where they were duplicated. The checks were more plentiful there since several hundred trees had been inoculated with *Diaporthe parasitica*. In a few cases we have found the western fungus spreading beyond the edge of the wound, i. e. apparently parasitic, but its development was so slow that it could be called at best, a weak parasite.

From these inoculation tests and from observations in the field, there is no longer any doubt but that the western fungus is a saprophyte and that it cannot develop into an active destructive parasite like *Diaporthe parasitica*. While we have not found it occurring in the same region, where the eastern fungus is common, yet the inoculations made at Charter Oak, show that it will not develop parasitic tendencies in a region where *Diaporthe parasitica* flourishes. Furthermore, by inoculations and by observations of natural infections, it has been proved that the true blight fungus develops normally about Connellsville.

CULTURAL COMPARISONS.

Shortly after isolating the western fungus, it was noticed that its development in culture was markedly different from *Diaporthe parasitica*. Further study of these differences resulted in securing cer-

tain kinds of media upon which the two fungi showed very marked contrasting characters. Both of these fungi produce conidial spore horns in much the same manner. If these spore horns are streaked on a potato agar slant, *Diaporthe parasitica* will produce an orange streak within four days at room temperature. This orange streak broadens, keeping pace with the growth of the fungus until the entire surface of the slant is covered with a deep orange growth. On the other hand no orange color is noticeable on the streak from the conidia of the Connellsville fungus even after a period of ten days. A lighter orange sometimes develops on these slants after a week or so but is never so marked as in *Diaporthe* and often fails to develop at all. Conidial streaks on other media, especially chestnut bark agar and corn meal agar, show a recognizable difference between the two fungi but this difference is not so marked as that of the color on potato agar.

On potato agar cultures from mycelial transfers a fan-shaped or irregular wavy growth is noticeable at the edge of the advancing mycelium in the case of *Diaporthe*, while the Connellsville fungus has an even unbroken edge. Furthermore, there is a marked contrast in the amount of aerial mycelium developed—*Diaporthe* developing scarcely any, while the Connellsville fungus has a fluffy appearance, due to a white mycelial growth above the surface of the agar. Also the contrast in color between the growths on potato agar is evident; especially in cultures about three weeks old. *Diaporthe* develops an orange brown color while the Connellsville fungus has at first a sulphur color which deepens as the culture becomes older.

Next to the conidial streaks on potato agar, we have found the growth on sterile twigs in test tubes to be the most accurate distinguishing character. The Connellsville fungus within ten days develops a fluffy orange mycelial growth which almost completely fills the tube. This mycelial growth is at first white, but turns to the orange color within a few days after its development. On the other hand, *Diaporthe* does not develop this heavy aerial mycelium but only a short white, web-like growth over the surface of the twig with heavier bunches of mycelium, which later become orange colored, where the pycnidia are to develop.

On the cut end of the twigs, *Diaporthe* develops a thick felt-like orange mycelial growth but this never extends out on the bark and is much denser than the growth of the Connellsville fungus. We have made these cultures on black oak, chestnut oak, white oak, chestnut, maple and sumach, but find very little difference in the

nature of the growth. We have used these tests on fungi from over fifty different sources and have never failed to get these characteristic reactions. These tests are always checked when possible by ascospore measurements and often by inoculation on live trees. No doubt many other cultural differences could be discovered by further tests, but these given have proved to be so reliable that no further effort was made to find media which would show additional differences.

In culture the fungi collected at various points in Virginia, West Virginia and Eastern Tennessee, show no variation from the Connellsville type of the fungus. This conforms with the results from the spore measurements. These fungi are evidently the same.

DISTRIBUTION OF THE CONNELLSVILLE FUNGUS.

Up to date this fungus has been found in Pennsylvania only in the four southwestern counties—Greene, Washington, Fayette and Westmoreland. Many specimens were examined from other parts of the State, which were thought to be the same, but in all cases they were found to be *Diaporthe parasitica*. The fungus probably occurs in other parts of the State but has so far not been reported. Since it was found as far down as the West Virginia line, visits were made over into this State and the same conditions were found there. Early in the investigation, it had been suspected that this Connellsville fungus was the same as that which had been reported from several points in Virginia. A visit to that State revealed the same condition of the chestnut timber as about Connellsville. As reported on a previous page the microscopic and cultural characters were found to correspond, so that there is no doubt as to the identity of the two fungi. Specimens were also sent, by Mr. J. K. Esser, from various parts of Eastern Tennessee, and these were found to be the same as the Connellsville fungus. As indicated by the collections then, we may say that this fungus is distributed throughout Southwestern Pennsylvania, West Virginia, Virginia and Eastern Tennessee. It is not at all improbable that further search will show that it occurs in several other States.

There is another fungus found in the extreme south—Florida, Alabama, South Carolina and Mississippi—which is very similar in external appearance both to the Connellsville fungus and to *Diaporthe parasitica*. This is the fungus found in Ellis and Everhart's N. A. Fungi (No. 1956), where it is labelled *Endothia gyrosa*. It is also found in a number of other North American collections under this name. The ascospores of this fungus measure 8.2×1.90 u, being much longer in proportion to their width than the Connellsville fun-

gus. They are cylindrical in shape and are very well represented in Ellis and Everhart's North American Pyrenomycetes. Besides the *exsiccati* we have also received specimens of this fungus from several points in North and South Carolina.

TAXONOMIC RELATIONS

What is the Connellsville fungus? There is no question but that it is very closely related to *Diaporthe parasitica* and should be placed in the same genus. Following Saccardo's system of classification it undoubtedly falls in the genus *Endothia* and fits well his description of *Endothia gyrosa*, in so far as the spore measurements and microscopic characters are concerned. It is certain, however, that it is not the same as the long-spored southern form.

The synonymy of *Endothia gyrosa* given by Saccardo is misleading since it is certain that Schweinitz and Fries had in mind two very different species when they wrote of *Sphaeria gyrosa* and *S. radicalis*. Furthermore, if the genus *Endothia* was founded by Fries on *Sphaeria gyrosa*, as Farlow (1.) believes it was, then the generic name *Endothia* is not correct when applied to this fungus—provided we go back to Fries (2) for the definition of the genus. Schweinitz (3) in 1822 described *S. gyrosa* as No. 24 of his *Syn. Fung. Car.*, but so far as we have been able to find, there is no specimen in the Schweinitz collections corresponding to the number of this description. It is probable that the specimens of this collection were included in his North American Fungi and in this collection there is a specimen of this species (No. 1431) which fits very accurately his description of *S. gyrosa* in *Syn. Fung. Car.* In fact, in looking at this specimen under a powerful lens one is struck with the extreme accuracy of his description and one cannot doubt that he had this or a similar specimen under his lens. This fungus is entirely different from *Diaporthe parasitica* or the Connellsville fungus and one would not think of placing it in the same genus or even in a related genus. The most noticeable character macroscopically is that the entire surface of the stroma is covered with very regular hemispheres (sphaerulae of Schweinitz.) The perithecia are enclosed in each of these separate sphaerulae and their walls do not differ in color from the surrounding stroma. No neck is evident and the ostiole is inconspicuous or wanting. The conspicuous black walls and long necks of the perithecia of *Diaporthe* are entirely lacking. The perithecia are entirely within the knobs or spheres of the stroma, i. e., they do not extend down into the stroma. The ascospores are often slightly curved and the septa very indistinct. The average size of

the ascospores was 15.82×4.37 u. The asci have very distinct walls and are not shaped at all like the asci of *Diaporthe*. They average 54 u. in length.

Schweinitz's *Sphaeria radicalis* (N. A. Fungi No. 1269), is an entirely different fungus from the above and although the perfect stage is not present, it resembles very closely the imperfect stage of the Connellsville fungus, or *Diaporthe parasitica* or the long-spored southern form. Any one who has worked with the above species will be convinced that Schweinitz (4) was writing of the perfect stage of one of these forms when he says, "*Ostioles cylindrical, very black within, orange red externally, everywhere elevated on the surface, easily falling off—whence the exposed surface shows black points, on account of the black shining ducts by which the ostioles are connected with the perithecia.*" But which of the three he had in mind would be hard to say unless the perfect stage is examined. Fries (2) places *S. gyrosa* in the tribe *Confluentes* and *S. radicalis* under the tribe *Versatiles*, thus widely separating the two, since the characters of these tribes are quite distinct. His descriptions of the two species follow closely those of Schweinitz. His distinction between the two is best brought out in his *Elenchus* (5) where, in describing *S. radicalis*, he says, "*A wonderful little fungus—certainly comparable only with S. gyrosa but very different from this in the position of the perithecia and the ostioles. Ostioles numerous, conical elongated, fragile, spinelike. Perithecia minute, black globose, sunken, also continuous through the spine-like ostiole by a little black duct.*" Under *S. gyrosa* he says, "*There is no distinct ostiole,*" and does not mention the beaks so noticeable in *S. radicalis*. This agrees well with the specimen of Schweinitz, where no ostioles are to be seen and the small knobs on the surface of the stroma contain simply the perithecia with no distinct necks.

It is, therefore evident that Fries had clearly in mind the distinction between these two species when he created the genus *Endothia* in 1846. In the meager and incomplete description he does not mention *S. radicalis* but he does mention *S. gyrosa* and it is to be presumed that he intended this species to be the type of the genus. Although he promised later to describe the characters of this genus more fully, we find no further mention of it in his later publications. If we admit that he used *S. gyrosa* as the type for erecting this genus and if we wish to include under it only species resembling it, then it is evident that the present *Endothia* is an entirely different genus from what Fries intended. As further evidence that he did not intend to place *S. radicalis* in this genus we may turn again to

his descriptions where he speaks of the perithecia being light colored (*pallidus*) and yet he distinctly mentions the dark walls of the perithecia in his description of *S. radicalis*.

In 1863, however, De Notaris (6) without any explanation, put the two in the same genus, and in the same year we find them combined by Tulasne (7) under the genus *Melogramma*. Since that time all authorities without further investigation have considered that Schweinitz gave these two names to one and the same species.

If the generic name *Endothia* is to be retained for those species resembling *S. radicalis* of Schweinitz and Fries and the more recently described *Diaporthe parasitica*, then we believe that the Connellsville fungus would fall in the genus *Endothia*. If, however, we wish to retain under this name such species as that on which Fries erected the genus, the Connellsville fungus would certainly not fall in this genus, and a new one will have to be erected to include *Diaporthe parasitica*, the Connellsville fungus and the long-spored southern form of Ellis and Everhart. According to our present system of classification, the form on which Fries erected the genus *Endothia*, would easily fall in a previously established genus and this name is now left without any significance whatever. Besides we are not certain that Fries meant to give a generic description in this short note since he states that he expects to describe the genus more fully later.

The simplest way out of this taxonomic tangle then, it seems, would be to retain the name *Endothia* for the forms such as Saccardo includes under it. Then we would have in our territory (1) the long-spored Southern *Endothia*, (2) the true blight fungus—*E. parasitica*—and (3) the Connellsville fungus, for which we proposed the name *E. Virginiana* and for which we have published a description.*

*Phytopathology 2:261-262, Dec. 1912.

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Fig. 1. Fans or mats of mycelium of chestnut blight fungus in the cambium and inner bark. Photo by E. T. Kirk.

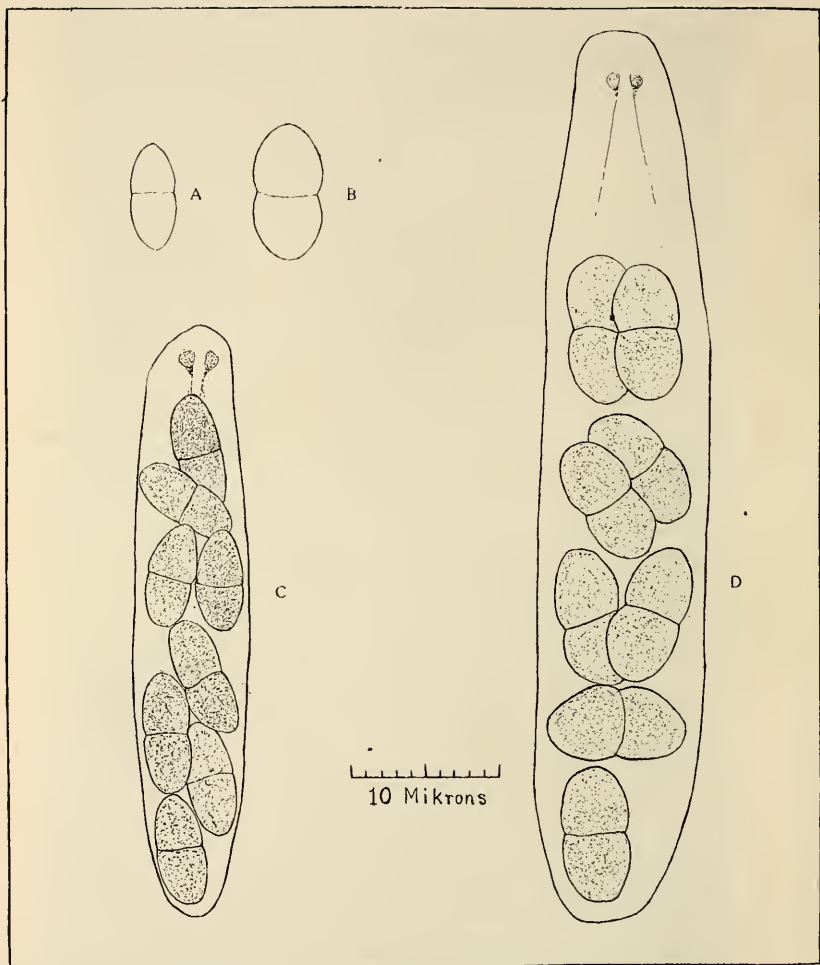


Fig. 2. A. Single ascospore of the Connellsville fungus. B. Single ascospore of the chestnut blight fungus. C. Ascus of the Connellsville fungus. D. Ascus of the chestnut blight fungus.



Fig. 3. Map showing the distribution of the Connellyville fungus in Pennsylvania. Drawn by J. K. Hibbs.

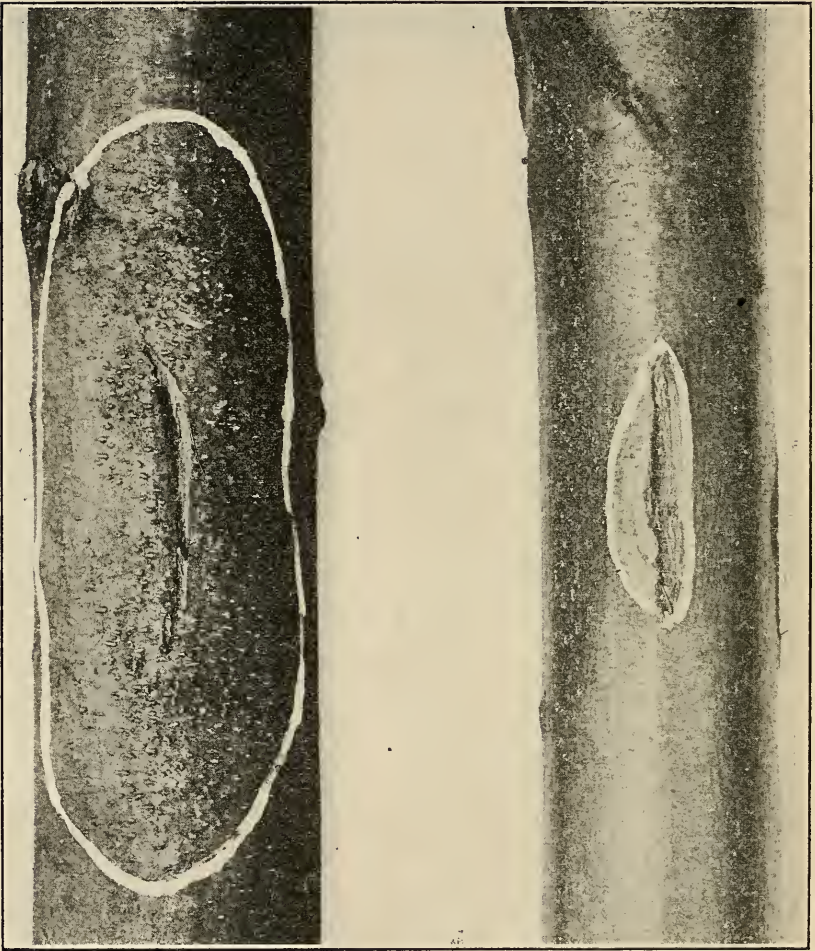


Fig. 4. Inoculations of 1 1-4 inches tree with Connellsville fungus, after five months (right); inoculation of 1 1-2 inches tree with blight fungus, after three months (left). Both outlined with paint to show extent of growth. Photo by E. T. Kirk.

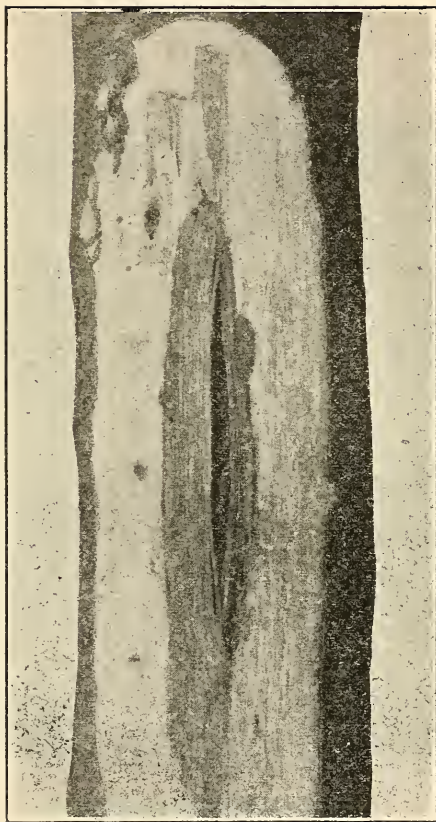


Fig. 5. Bark cut away from inoculation with the Connellsville fungus, showing the even outline. Photo by E. T. Kirk.

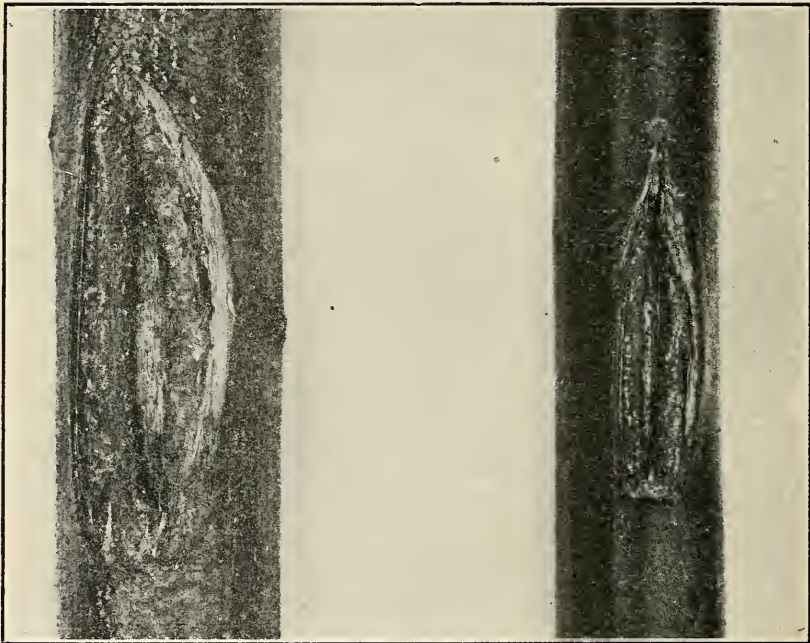


Fig. 6. Two inoculations with the Connellsville fungus showing callus formed around each. Trees 1 inch and 1 1-2-inches in diameter. Photo by E. T. Kirk.

PENNSYLVANIA CHESTNUT TREE
BLIGHT COMMISSION

1112 Morris Building, Philadelphia

Bulletin No. 5

MAY 15, 1913

The
Symptoms of Chestnut Tree Blight
and
A Brief Description of the
Blight Fungus

by
F. D. Heald, Pathologist



1913

Pennsylvania Chestnut Tree Blight Commission

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Introduction

INQUIRIES are constantly being received by this Commission for more detailed information about the chestnut tree blight fungus. On the other hand, certain erroneous ideas exist in regard to the nature of this fungus. This circular is written with the hope that it will supply some desired facts and assist in correcting false notions. Investigations on the dissemination and life-history of the blight fungus are in progress at the present time and new facts are constantly being determined. Our knowledge being far from complete, it is only advisable to present the facts which appear to be fairly well established.

Symptoms and Effects

Young infections of chestnut blight on smooth-barked vigorous shoots (two to six or more years old) can be easily recognized by the presence of yellowish or yellowish brown patches, slightly raised, and standing out in marked contrast to the olive-green healthy bark. The area invaded by the fungus may be fairly regular (Figs. 4, 23) or very irregular in outline, the latter showing what has been designated as the amoeboid type (Figs. 1, 2, 3). In young infections of this type there are no fruiting pustules, but these make their appearance later. If the external brown layer of cork cells is removed from the advancing edge of the invaded area, the whitish or buff-colored mycelium, or vegetative body, of the blight fungus is exposed (Fig. 24). Infections of this type may spread until the shoot is completely encircled (Fig. 4), and fruiting pustules will be formed later.

Young infections on slow-growing twigs or on the smooth bark of older branches or trunks are not as evident, but they generally show as somewhat discolored, dead areas, sometimes slightly depressed, and occasionally with a raised margin. The area invaded may be nearly circular, giving a so-called "target" infection, but it is more frequently elongated in the direction of the long axis of the shoot or branch. The invaded area gradually enlarges until the shoot or

branch is completely encircled. A small shoot may be completely encircled before the appearance of fruiting pustules, but on larger limbs or on the main trunk the fruiting pustules begin to make their appearance long before complete girdling has taken place. These fruiting bodies show as small yellow, orange or reddish brown pustules ($1/16$ inch or slightly more in diameter) which break through the bark some distance back from the advancing edge of the lesion.

The interior tissue (inner bark invaded by the fungus) is changed to a yellowish brown color, which is in marked contrast to the bright fresh color of the normal healthy tissue, and a careful examination by cutting away the bark will show the buff-colored fans of the fungus which may have penetrated as deep as the cambium layer (Fig. 12).

During damp weather following rains, or in moist situations, long, irregularly twisted threads varying in color from buff to bright yellow may be extruded from some of the pustules (Fig. 13). These are masses of conidia or summer spores, and have been designated as "spore-horns" or tendrils. The spore-horns when first formed are soft and sticky, but when dry they become hard and brittle and are frequently darker in color.

Young infections on old trunks or large limbs with thick fissured bark cause little change in the appearance of the bark itself and the fungus may have gained considerable headway before there is any external evidence of its presence. Sometimes the first indication of an infection on large limbs or trunks is the appearance of abnormal longitudinal splits or fissures. The orange or yellow fruiting pustules appear in the deep crevices or cracks, and spore-horns may be developed from these under favorable conditions of moisture and temperature. In case of doubt as to whether a given discoloration is caused by the blight fungus the following test may be used: Place the twig or piece of bark in a closed vessel so it is supplied with plenty of moisture and will be retained in a moist atmosphere. In all cases if the fungus is present and is alive, bright yellow or orange,

cottony tufts will make their appearance upon the surface, and in many cases spore-horns will also be developed.

An infection with the blight fungus is sometimes the cause of a pronounced enlargement, or hypertrophy. This enlargement may involve the entire invaded portion (Figs. 6, 7) or it may be more pronounced at the upper end of the lesion (Fig. 5). Enlarged lesions are apparently the most frequent on vigorous shoots. Longitudinal splits or fissures in the bark are very characteristic of hypertrophied lesions (Fig. 7). In many instances the lesion may show a marked sunken area (Fig. 8) due to the killing of the invaded bark, while the surrounding tissues have continued to grow at the normal rate. This dead tissue may be more or less cracked or fissured and a typical canker developed (Fig. 8). In the old lesions which have completely girdled a limb or branch the bark becomes cracked and fissured and begins to peel away (Fig. 9). The branch shown in the cut referred to had been killed by this lesion and had been dead for a year. On old rough-barked trunks or branches the bark over old lesions will give a hollow sound when tapped, due to the fact that the inner bark has been destroyed by the fungus. The bark may be readily peeled away and the inner fibrous portion is more or less shredded.

Aside from the discovery of the actual lesions there are various other symptoms which indicate the presence of blight. *Dead leaves* hanging in characteristic drooping clusters are an indication of blight-killed twigs or branches. If the twigs or branches were not killed until late spring or summer, that is, prior to the first of September, the leaves reach normal size, and these clusters of dead leaves will generally remain clinging to the tree during the winter period after the normal leaves have fallen. This affords one means of detecting blight-killed branches in the winter. In blight-affected branches there is an indirect effect upon the *size and persistence of the burs*. If the girdling is completed early in the growth of the burs, they are likely to remain small and undersized, but with later completion of girdling they may attain full size. These burs of blight-killed

branches commonly remain hanging upon the tree during the winter, constituting another evident symptom for the detection of blight during the leafless period.

In case the girdling of a branch is not completed until late fall, the normal shedding of the leaves occurs. In the spring, however, the leaves from these branches remain undersized and assume a yellowish or pale color, and soon wither and die (Fig. 11). If girdling is completed later in the spring or not until midsummer, the leaves of the affected branches develop to full size, but later turn yellowish or assume a characteristic reddish brown color. Later when the leaves die they assume more of a brownish tinge, and some fall from the tree while many remain hanging for a considerable time.

The development of sprouts or "suckers" is another evident symptom of blight which can be noted at any period in the year. As soon as a branch or the main trunk has been girdled by blight, there is a marked tendency to the production of vigorous, rapid-growing shoots from a point just below the girdled area. These sprouts may be few in number or they may be so numerous as to make a conspicuous clump (Fig. 11), and they may occur on the branches, the main trunk, or at the base of the tree. These sprouts may be killed in turn by the blight, but they sometimes persist for several years. When they persist their age serves to tell the time at which the girdling was completed. The general effect of blight is to kill the part of twigs or branches beyond the lesion. The occurrence of trunk lesions is most serious, since with the completion of girdling the entire tree must succumb. In trees which have suffered from top infections for several years, the occurrence of the blight-killed branches sometimes gives rise to an effect called "stag-head." The wood of blight-killed trees is injured but little as a direct result of the disease, but if left standing it soon begins to deteriorate as a result of the work of insects and various species of wood-destroying fungi.

The Blight Fungus

The chestnut blight is due to a definite species of fungus which grows as a parasite in the bark and to some extent in the wood of the infected tree. This fungus was first described as *Diaporthe parasitica* Murrill, but has since been referred to *Endothia parasitica* (Murr.) And. It is possible to grow this fungus in artificial cultures (Fig. 25) and it has been repeatedly demonstrated by inoculations into healthy trees to be the cause of the disease.

1. *The vegetative body or mycelium.* The blight fungus grows within the bark and to some extent in the wood of the affected parts, where it produces strands or mats of closely appressed filaments, known as the mycelium or vegetative body of the fungus. In young infections on smooth-barked shoots this mycelium is located just below the brown, outer, or corky bark, and is cottony white at the advancing edge but assumes a buff tinge in the central or older portions of the infection (Fig. 24). As the infections become older, the mycelium penetrates deeper and spreads out at various depths in the bark, where it produces characteristic fan-like aggregates. The fans of buff or yellowish mycelium are especially well developed in the layers of inner bark, and finally in the cambium or growing layer between bark and wood, which is thus destroyed by the growth of the fungus (Fig. 12). After the mycelium has reached the cambium and spread out in that region, it enters the wood and grows throughout the outer layers of sapwood. It is known to penetrate at least as far as five annual rings of wood.

2. *The pycnidial stage.* After the mycelium of the blight fungus has been growing for a time in the bark it begins the formation of fruiting pustules for the production of spores. The first kinds that are produced are known as *pycnidial pustules* or stromata, and they appear as minute raised papillæ scarcely larger than a pin-head, and showing a yellowish or orange color when they break through the bark. Each pycnidial pustule shows a smooth or slightly uneven outer surface and is a dense aggregate of fungous tissue, generally containing one (rarely more) large, lobu-

lated cavity (Fig. 14) lined with innumerable vertical filaments by which large numbers of minute rod-shaped bodies, the pycnospores, are produced. With the accumulation of these in a pycnidium, the external wall is ruptured and the accumulated mass of spores imbedded in mucilaginous material oozes out in the form of a thread-like or flattened irregular coil, the so-called "spore-horn" or tendril (Fig. 13). A single spore-horn of average size has been found by actual analysis to contain as many as 115,000,000 pycnospores (Fig. 21).

The pycnospores have frequently been designated as summer spores, but the development of pycnidia depends largely upon the age of the lesion rather than on the time or season of the year. Pycnospores are produced in abundance at all times in the year when temperature and moisture conditions are favorable, and are washed down in large numbers from diseased branches even during the warm winter rains, when the spore-horns are rarely observed.

The production of pycnospores is not confined to pustules which break through the bark of diseased areas. Smaller orange or reddish superficial pycnidia may be produced in large numbers on the cut end of the inner bark or the outer layers of sapwood (Fig. 18) of fallen logs, stumps, or wood previously affected with blight, or on the inner surface of inner bark where it has split away from the wood. Peeled posts and poles previously affected with blight will frequently show many of these minute pycnidia on the diseased spots, but these pycnidia are generally rather scattered. Pycnidia producing large numbers of viable spores have been obtained from a wood-pile two years old. Chips or fragments of diseased bark or wood that fall in damp locations will produce pycnidia, so that material of this sort is always a possible source of infection.

3. *The perithecial stage.* Following the production of pycnidia and pycnospores, a second type of fruiting pustules containing the perithecia makes its appearance. Superficially these perithecial pustules can be readily differentiated from the pycnidial pustules, since each one shows upon

its surface either a number of minute raised papillæ or a number of minute black dots, the ostioles or openings of the *perithecia* or flask-like bodies buried deep in the stroma (Figs. 16, 17).

Each perithecial pustule is a dense aggregate of fungous tissue containing 1 to 60 distinct flask-like cavities, the perithecia, each of which communicates with the exterior by means of a long black neck which opens at the top of a surface papilla (Fig. 15). The wall of each perithecium is lined with small club-shaped cells or spore-sacs, which are produced in enormous numbers (Figs. 15, 20) and give rise to the second type of spores or ascospores. There is one perithecium for each superficial papilla.

The perithecial pustules show some differences in color and external appearance depending upon their age and the conditions under which they have developed. The papillæ and the stroma may both be yellowish or orange, or the papillæ may be yellowish brown to brick-red on a lighter ground, or in old pustules the stroma may be nearly black, with slightly lighter papillæ. In most cases when the perithecia are mature the ostioles or mouths of the necks will show as dark spots at the ends of the surface papillæ. There is considerable variation in the length of the surface papillæ, the difference being due to varying amounts of moisture, those which develop with an abundance of moisture showing especially long necks, while with scarcity of moisture the papillæ remain short.

The spore-sacs formed in the perithecia contain the ascospores. Each sac produces eight two-celled spores arranged generally in two irregular rows (Fig. 20). These spores have a volume about fifty times as great as that of the pycnospores (Fig. 21). They are not extruded ordinarily in masses from the perithecia, but under favorable conditions of moisture and temperature the spore-sacs rise to the ostiole and explode, forcing the spores into the air. If a glass slide is suspended 1/8 inch or slightly more above the surface of some mature perithecial pustules moistened in water and kept at a temperature not under 65° F., large

numbers of ascospores will be expelled and will adhere to the slide. Such a spore print of ascospores is shown in Fig. 22. A similar expulsion of ascospores takes place in nature whenever conditions are favorable.

The ascospores have been designated as "winter spores." Their time of maturing, however, appears to depend more upon the age of the lesion than upon the season of the year. Maturing perithecia may be found at any season of the year, although they are perhaps more abundant in the fall and winter than at other seasons. Successive crops of perithecial pustules may be found on a single lesion which has persisted for a number of years. The blight fungus may spread throughout the bark of a blight-killed tree and continue to produce fruiting pustules, or perithecial pustules may be produced in abundance in the crevices of the bark of fallen logs (Fig. 17).

The Spread of the Disease

The cause of infections. New infections, whether in sound trees or in those already diseased, are caused by the establishment of the vegetative body or mycelium of the fungus in the tissues. This mycelium originates from either pycnospores or ascospores. Successive stages in the germination of both kinds of spores are shown in Fig. 26. If this germination takes place in some wound which penetrates the outer brown bark, the fungus readily establishes itself and begins to grow through the tissues of the bark in much the same way that it is growing in the culture medium shown in Fig. 25. An infection can be caused then by either a single ascospore or a single conidiospore if they are carried and lodged in a favorable location. A large percentage of the new infections appear to be definitely related to some mechanical injury, but there are some evidences that natural cracks and fissures may also be the avenue of entrance.

Natural agencies in dissemination. The pycnospores or the ascospores must be carried from one part of a tree to other parts, or from tree to tree, if new infections are to result. Present investigations point to the fact that asco-

spores which are forcibly expelled into the air during the moist and warm periods of the year play a very important part in the spread of the disease, since they can be carried by the air currents. It can also be definitely stated that conidiospores are washed down during every rain, even the cold rains of winter, in countless numbers from every lesion that has reached the spore-producing stage.

It seems probable, then, that conidiospores play a very important part in the spread of the disease throughout a tree after it once becomes infected. Rain and wind are undoubtedly the most important natural agents in the dissemination of spores.

The part which birds, insects and other animals play in the scattering of spores is at the present time somewhat problematical. The few tests reported up to date have given only negative results. (See Bulletin No. 3 of the Pennsylvania Chestnut Tree Blight Commission.) From investigations now in progress it may be definitely stated that a single downy woodpecker has been found to be carrying as many as 657,000 pycnospores.

Artificial agencies. It has been definitely shown in numerous cases that the shipment of infected chestnut nursery stock has been responsible for the introduction of blight into a new region. After it is once introduced, natural agencies may be responsible for the scattering of the spores.

The shipment of chestnut products of various kinds, such as logs, wood, posts, poles etc., made from blight-affected trees may also be responsible for spreading the disease, since the mycelium may retain its vitality in blighted bark or wood for long periods and produce new crops of pycnidia very soon after moisture is supplied, or spores may be scattered from pustules formed previous to shipment of the products. (See also Bulletin No. 3.)

Explanation of Plates

All photographs are by Wm. Currie, except Fig. 12, which was made by E. T. Kirk.

PLATE I

Fig. 1. Amœboid infection on two-year-old shoot. Bark has been removed and spread out flat.

Fig. 2. Amœboid infection on three-year-old shoot.

PLATE II

Fig. 3. Characteristic amœboid infection on two-year-old shoot.

Fig. 4. Basal infection on two-year-old shoot. The fungus has completely encircled the shoot.

PLATE III

Fig. 5. Characteristic hypertrophy of two-year-old shoot.

Fig. 6. Characteristic hypertrophy of two-year-old shoot.

PLATE IV

Fig. 7. Characteristic hypertrophy of vigorous shoot.

PLATE V

Fig. 8. Lesion that nearly surrounds the branch. Sunken on one side, and a slight enlargement on the other.

PLATE VI

Fig. 9. Old lesion in which the bark has become somewhat shredded and the wood exposed. The branch had been dead for a year.

PLATE VII

Fig. 10. Characteristic position of drooping leaves on blight-killed shoots. Shows also a small bur.

- Fig. 11. Water sprouts produced at the base of a tree recently girdled by the blight fungus. Tree also shows few small leaves, giving the characteristic appearance of a blight-killed tree.

PLATE VIII

- Fig. 12. Fan-shaped mycelium from bark of a rough-barked tree. (After Anderson.)

PLATE IX

- Fig. 13. Pycnidial pustules with spore-horns developed in a damp chamber in the laboratory.

PLATE X

- Fig. 14. Vertical section of a pycnidial pustule. The filaments lining the lobulated cavity produce the spores that ooze out as "spore-horns."
- Fig. 15. Vertical section of a perithecial pustule. Several of the perithecia are cut so as to show the full length of the necks.

PLATE XI

- Fig. 16. Perithecial pustules enlarged (x 3).
- Fig. 17. Perithecial pustules in the crevices of rough bark. From a fallen log.

PLATE XII

- Fig. 18. Pycnidia on the end of a fallen log. Three zones are shown, one for each of the three outer rings of wood.
- Fig. 19. Vertical section of pycnidia shown in Fig. 18.

PLATE XIII

- Fig. 20. Spore-sacs or asci, each containing eight spores.
- Fig. 21. Diagram showing relative size of pycnosporos (left) and ascospores (right). Maximum and minimum sizes of each are shown.

PLATE XIV

- Fig. 22. Photograph of an ascospore print on an object slide. Made by inverting a slide over perithecial pustules that have been soaked with water and kept for a time at a temperature favorable to the expulsion of ascospores.

PLATE XV

- Fig. 23. A young lesion of the chestnut blight fungus on a vigorous two-year-old sprout.
- Fig. 24. The same lesion as above with the brown outer bark removed to show the white or buff-colored mycelium.
- Fig. 25. Isolation culture made from the above lesion before the removal of the bark. A minute portion of the mycelium was planted at three different spots in the culture plate.

PLATE XVI

- Fig. 26. Photograph showing successive stages in the germination of both kinds of spores. (a) ascospores series from 8 to 22 hours, at hourly intervals; (b) conidiospores series from 8 to 22 hours, taken every two hours.



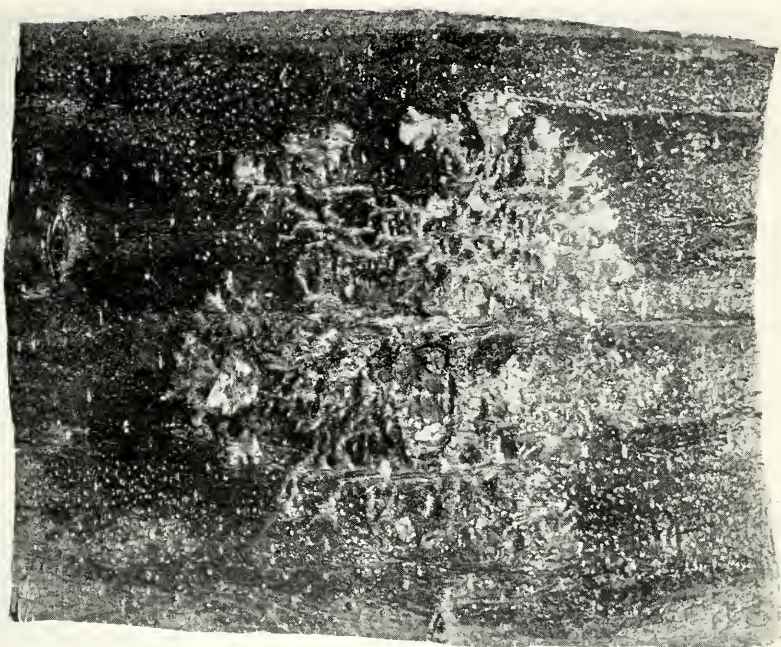


Fig. 1

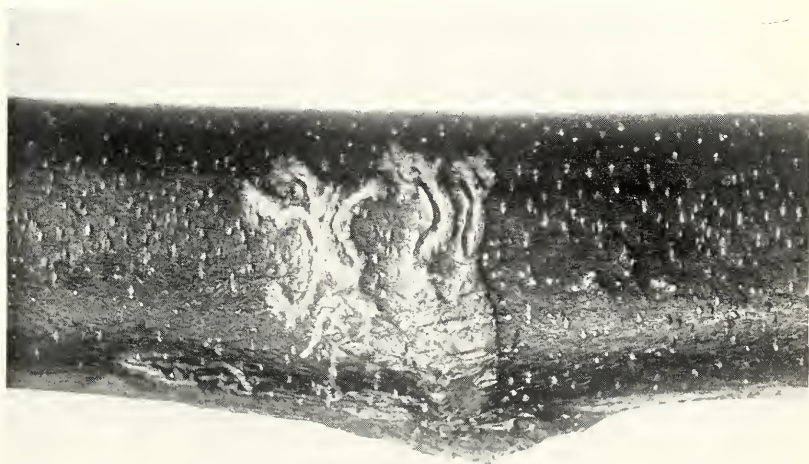


Fig. 2

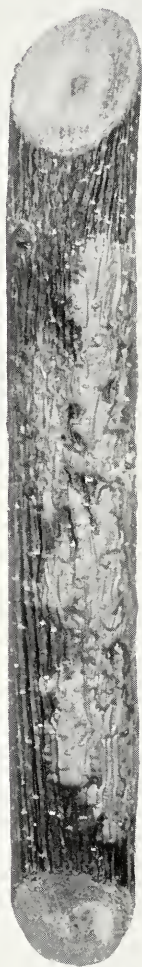


Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7



Fig. 8



Fig. 9



Fig. 10



Fig. 11



Fig. 12

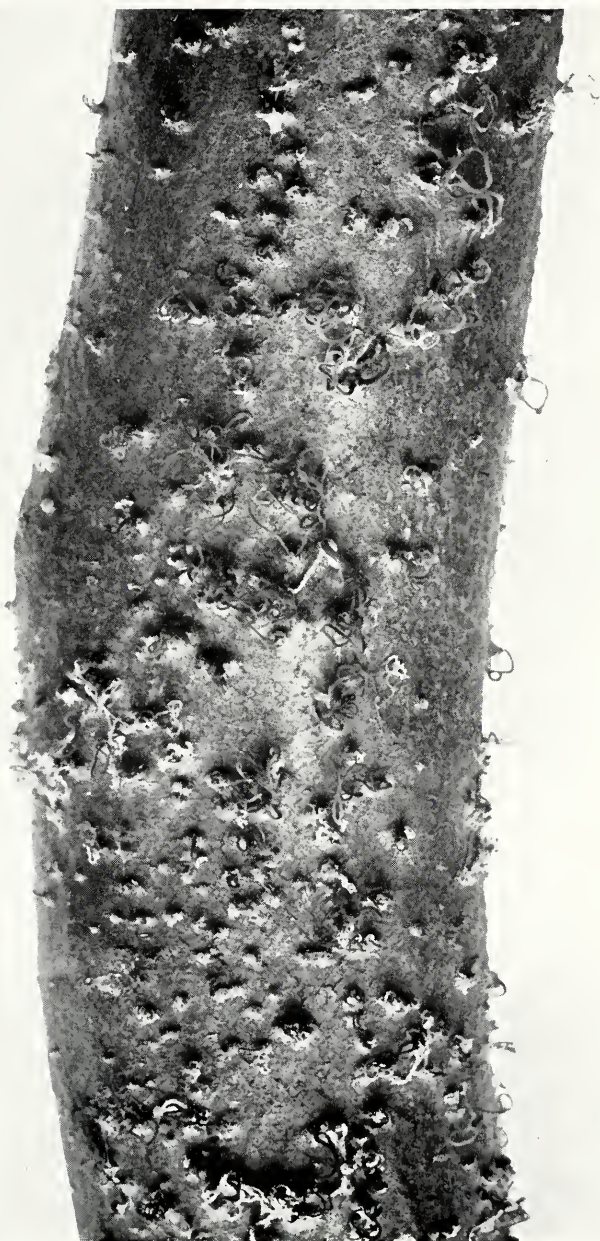


Fig. 13

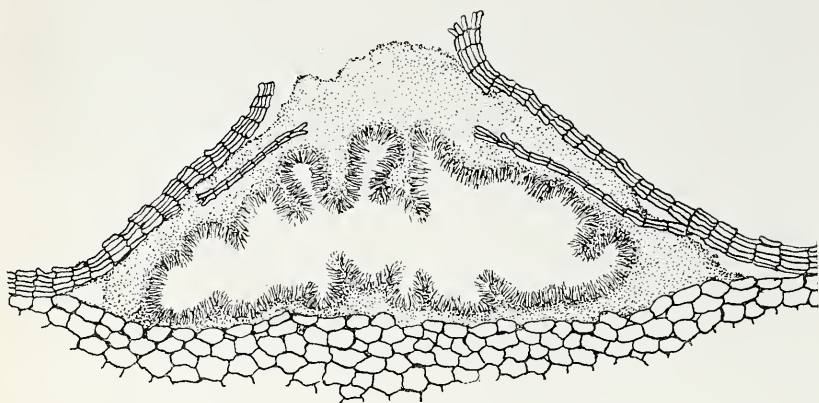


Fig. 14

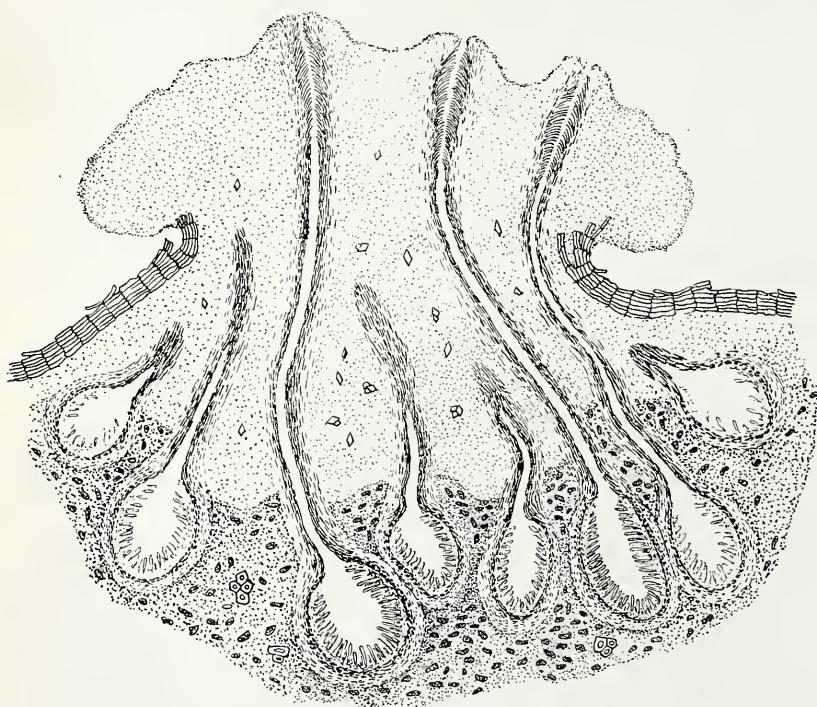


Fig. 15



Fig. 16



Fig. 17

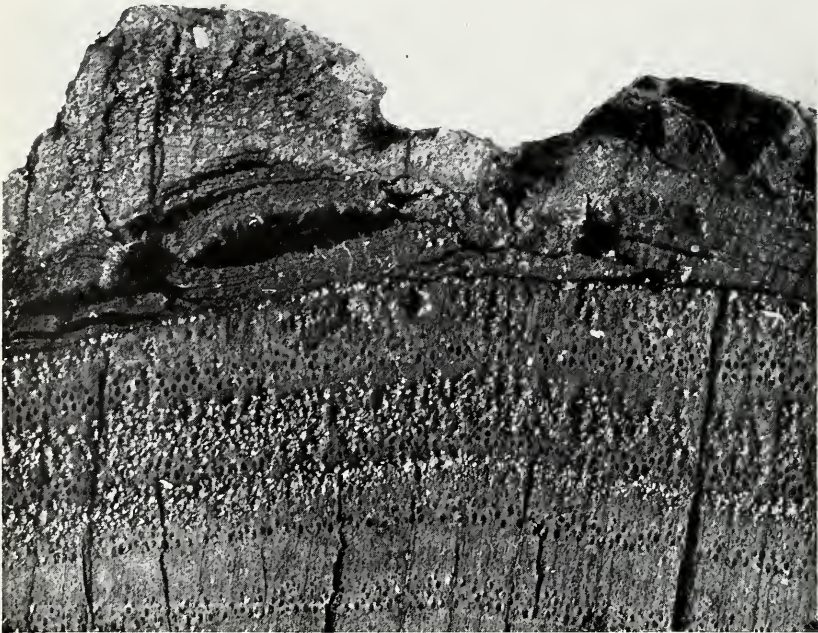


Fig. 18



Fig. 19

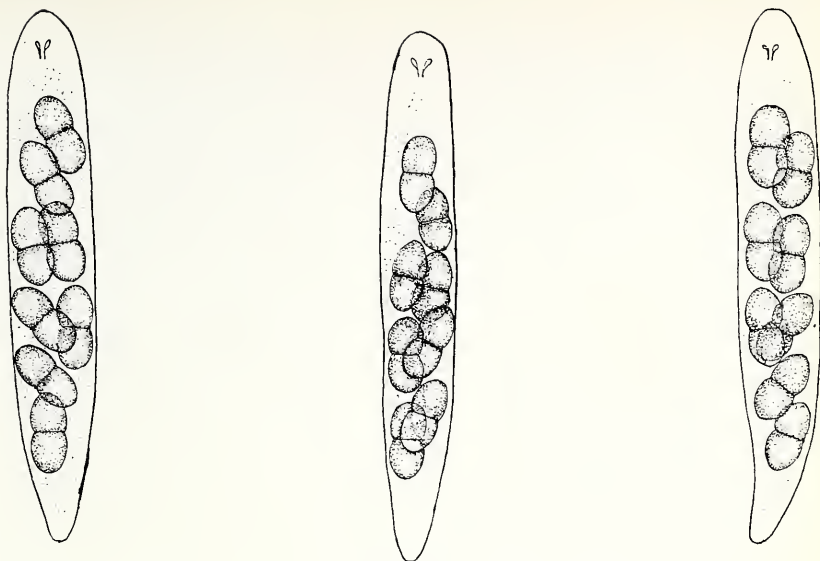


Fig. 20

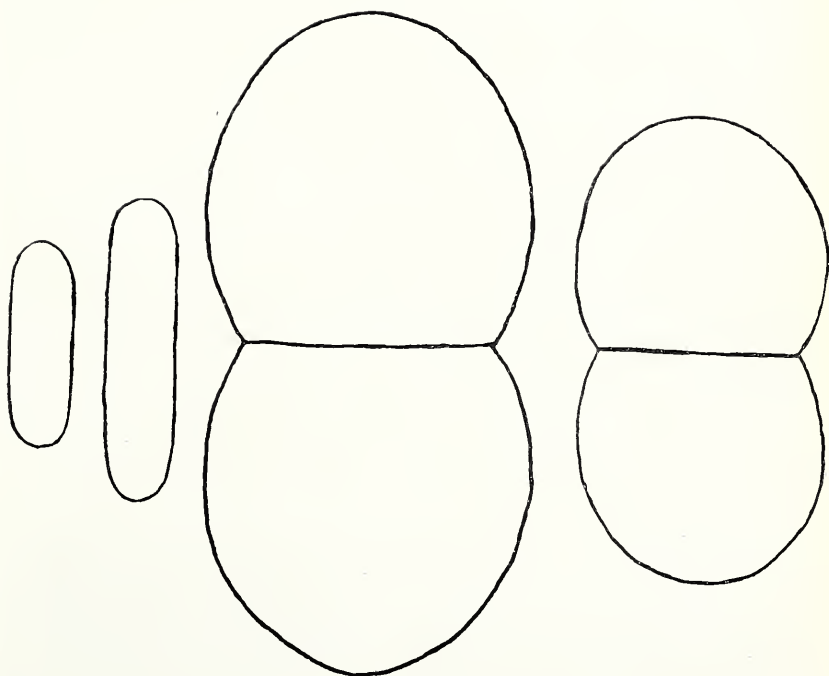


Fig. 21

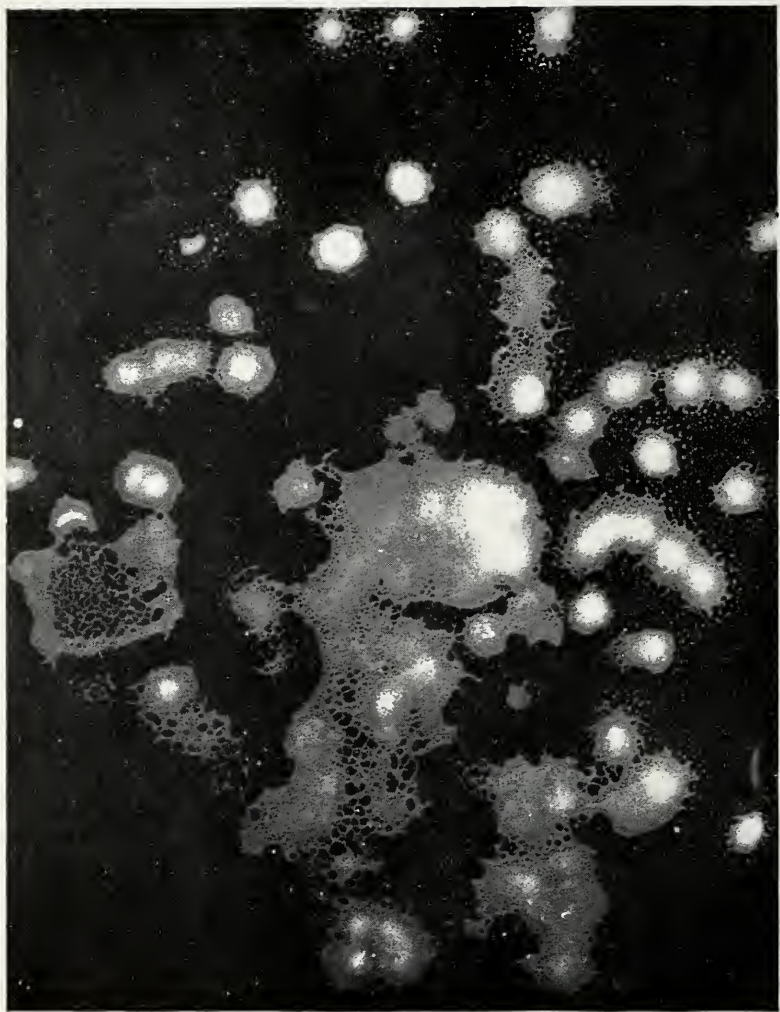


Fig. 22



Fig. 23



Fig. 24



Fig. 25

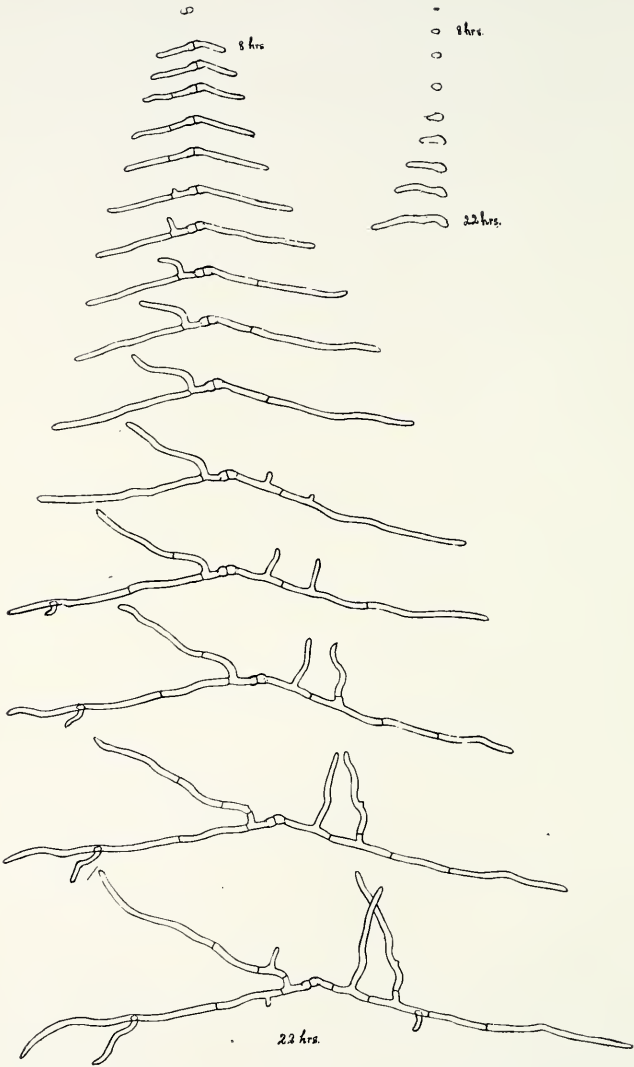
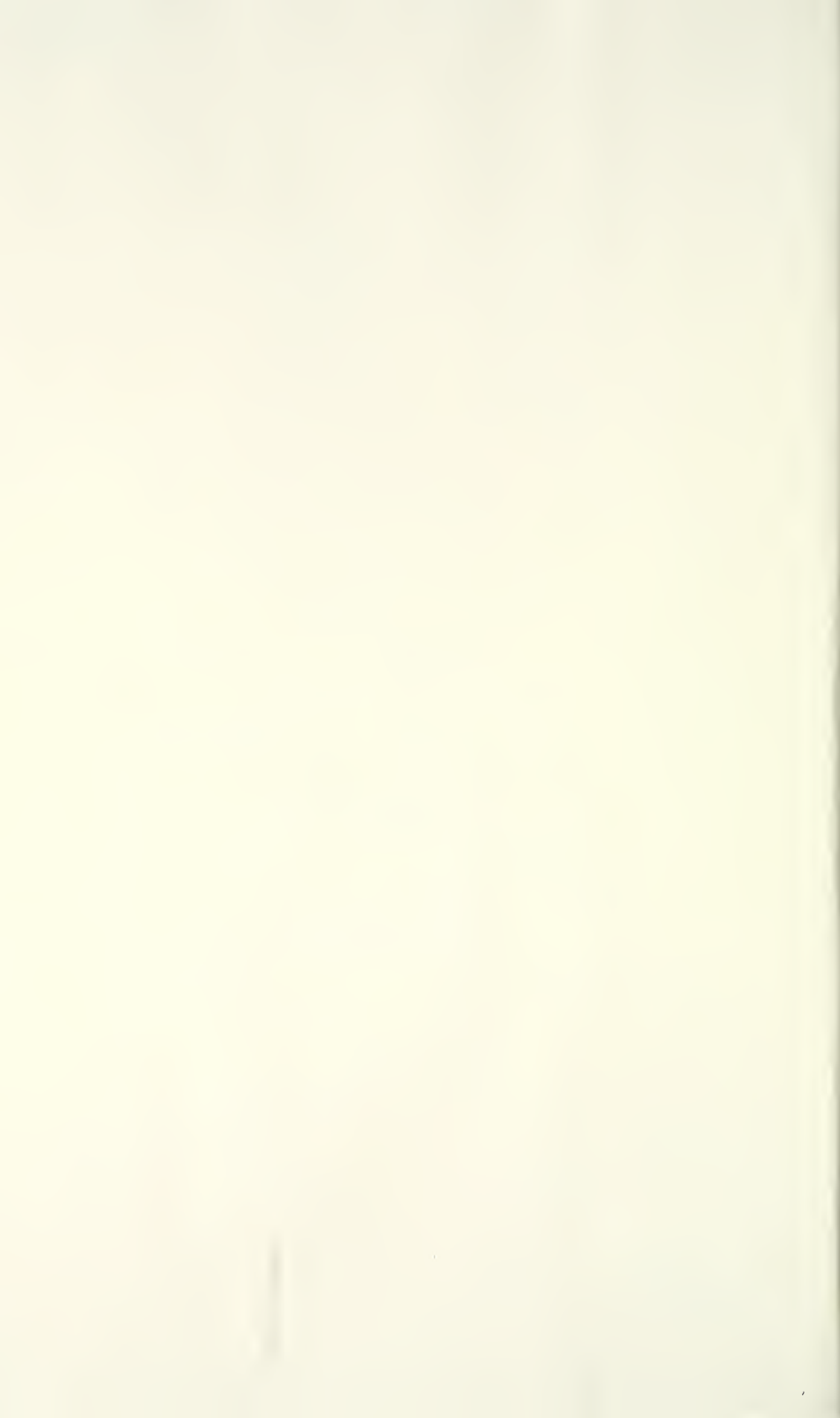
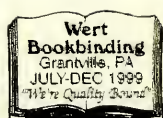


Fig. 26a

Fig. 26b







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